Herb – drug interaction of noni juice and Ginkgo biloba with phenytoin

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A B S T R A C T

The common perception regarding the herbal medicines is that they are natural and safe. Although considered natural, most of the herbal medicine can interact with other drugs causing either potentially dangerous side effects or they can lead to loss or decreased therapeutic benefits of the drugs. Currently, there is growing concern to analyse and understand the herb – drug interactions. This study investigates effects of noni juice/Ginkgo biloba on the pharmacokinetics of phenytoin and also on the oxidative stress associated with long term administration of phenytoin. After pretreatment for 7 days with noni juice and G.biloba, on day 8 the phenytoin was co-administered orally with noni juice and G. biloba and the serum pharmacokinetics were determined at various time points (1, 2, 4, 6, 8, 12 and 24 h) by HPLC. The oxidative stress markers were determined after 30 days of treatment. Noni juice pretreated rats decreased the bioavailability of phenytoin by 2.81 fold, whereas G. biloba pretreated rats increased the bioavailability by 2.08 fold when compared with control. Noni juice and G.biloba treated rats provided significant protective effect against the oxidative stress induced by long term administration of phenytoin. It is observed that noni juice and G.biloba might have altered the bioavailability of phenytoin due to induction and inhibition of CYP2C9 enzymes.

Key words: Noni juice, G.biloba, Epilepsy, Herb – Drug interactions, Phenytoin; CYP2C9.

INTRODUCTION

Noni plant, Morinda citrifolia L. (Rubiaceae) is a small tropical evergreen shrub or tree indigenous to the Pacific Islands, South-east Asia, and various other tropical and temperate areas. All parts of the plant and especially fruits are used in herbal medicine by Polynesians for the past 2000 years.[1] According to traditional uses and based on recent scientific research, the noni plant has been proven to have broad range of therapeutic effects including antibacterial, antiviral, antifungal, anthelmenthic, analgesic, anti – hypertensive, anti – inflammatory, and immune enhancing effects.[2] Studies also reveal that it has anti-tubercular,[3] anti-malarial activities.[4] The fruits are also known to have anti – HIV activity[5] and anticancer activity.[6] Noni is known to contain 3,3´ - bisdemethylpinoresicnol, americanol A, americanin A, amercanisic acid A, morindolin, isopincepin, and dammcanthal (anthraquinone) as the active constituents.[7-8]

Ginkgo biloba L. (Ginkgoaceae) has been used for a very long time in traditional Chinese medicine. The standardized extract of G.biloba is referred to as EGb 761, which contains 24% flavonoids (including monosides, biosides, and triosides of quercetin, kaempferol, isorhamnetin and 3´-O-methylmyristicos), 6% terpenes (including the diterpenes like ginkgolide A, B, and C and the sesquiterpene bilabolide), organic acids (kynurenic acid, 6-hydroxykynurenic acid, vanillic acid, shikimic acid, glucuric acid) with trace amount of ginkgolic acids (0.0005%), minor constituents like proanthocyanidins, glucose and rhamnose.[9-14] Bridi et al. 2001 reported that the antioxidant activity of G. biloba is due to flavonoid constituents and terpenoids are responsible for promotion of circulation.[15] G. biloba extract helps to protect neuronal membranes, facilitates better arterial circulation and improves electrical transmission in the brain.[16-18] G. biloba has beneficial effects on impaired brain glucose metabolism of streptozotocin – damaged rat brains, delays the progression of dependency and need for care in dementia patients.[19-21] G. biloba is reported to improve the decreased peripheral immune functions, schizophrenic symptoms, has stabilizing effect on the vestibular system.
of the ear[22-24] and there are also several reports on its anticancer activity.[25-32]

Deficiency of antioxidant status has been implicated in most of the neurological disorders and excessive lipid peroxidation is associated with teratogenicity and aggravation of seizures in epileptic patients.[33-36] Phenytoin and carbamazepine are the commonly prescribed antiepileptic drugs and studies depict that these antiepileptic drugs decrease antioxidant status in the body.[37-41] Studies have also revealed that phenytoin mono therapy leads to increased oxidative stress in the female epileptics.[82] In the pretext of avoiding the risks associated with oxidative stress, epileptics may tend to use the nutritional supplements rich in antioxidants.

The phenomenon of French paradox has drawn the attention of vast majority of people towards the beneficial effects of grape fruit juice and other functional foods. Similarly the use of fruit juices like orange juice, noni juice, star fruit juice and pomogranate juice etc., is increasing worldwide. Globally more than 200 companies are engaged in the commercial selling of noni juice and its products. Noni is currently distributed in more than 50 countries across the world, and its health benefits have been realized by millions of consumers.[43] Noni juice with its well balanced nutritional supplements containing vitamins, many trace minerals like calcium, magnesium and number of phytochemicals can act as a good antioxidant. It can enhance intercellular electrical signals in epileptic patients, which can prevent the seizure attacks and it can be the best choice to epileptics as an antioxidant. G. biloba extract is one of the most popular herbal medicines in the world which is known for improving cerebral circulation and cognitive functions.[44] Apart from its therapeutic use Ginkgo is also considered to be one of the important functional foods due to its rich flavonoid content. Noni juice and G. biloba can be used as alternative remedies for specific conditions like oxidative stress. These two herbal medicines i.e., noni juice and G. biloba were chosen for the present study in view of their rapid use as nutraceuticals and functional foods.

MATERIALS AND METHODS

Materials

The samples of Phenytoin and Carbamazepine were supplied as gift samples by Jubilant Organosys (Noida, India). Diclofenac sodium and Chlorozoxazone were kind gift from Dr.Reddy’s Laboratories (Hyderabad), Noni Juice (Cyber Noni) (Noni Connection Inc, USA) was purchased from the local dealer. G-KOBA (Ginkgo biloba) tablets were purchased from a local pharmacy, manufactured by Lessac Research Laboratories, Pondicherry, India. Each tablet of G-KOBA contained 40mg of dried extract of G. biloba (containing 9.6mg of ginkgoflavon glycosides). Ethyl acetate (Merck), Glacial acetic acid (Merck), Methanol (Merck) were purchased from the market. The study protocol was approved by the institutional animal ethical committee of Kakatiya University, Warangal, India. The HPLC was performed on LC – 10AT (Shimadzu Corporation, Kyoto Japan) system by injecting 20 µl of sample using Hamilton syringe (Hamilton Bonaduz AG, Switzerland) into syringe loading sample injector (Model 7725i, Rheodyne LP, CA, USA), diode array detector (Shimadzu SPD M10A vp model, Shimadzu Corporation, Kyoto, Japan), Biofuge Fresco centrifuge (Heraceus, Germany), cooling centrifuge (Remi Instruments, Mumbai, India), cyclomixer (Remi Instruments, Mumbai, India).

Experimental design

Noni juice/G. biloba – phenytoin interaction study

Male Wistar rats weighing 250 – 300 g were used for the study. Animals were maintained on regular rat feed of the animal house. The rats were divided into five groups of 6 animals each. The group 1 served as control. The group 2 was pretreated with 5ml/kg of noni juice for 7 days. The group 3 was pretreated with 100mg/kg of aqueous extract of G. biloba for 7 days. Group 4 was pretreated with 50mg/kg of fluconazole as CYP2C9 inhibitor for 7 days and the group 5 was pretreated with 50mg/kg of rifampicin as CYP2C9 inducer for 7 days. The rats were fasted for 16 hr prior to the study with water ad libitum. On day 8 the group 1 was administered orally phenytoin (20mg/kg suspended in 0.5% sodium carboxymethyl cellulose). Group 2 was administered orally with 5ml/kg of noni juice followed by phenytoin (20mg/kg suspended in 0.5% sodium carboxymethyl cellulose) after gap of 30 minutes. Group 3 was administered with 100mg/kg of aqueous extract of G. biloba followed by 20mg/kg of phenytoin after a gap of 30min. Group 4 was administered with 50mg/kg of fluconazole followed by 20mg/kg of phenytoin after a gap of 30 minutes. Group 5 was administered with 50mg/kg of rifampicin followed by 20mg/kg of phenytoin after a gap of 30 minutes. Blood samples were withdrawn by the retro - orbital vein puncture at different time intervals, viz. 0, 2, 4, 6, 8, 12 and 24 hours. The hypovolaemic condition was prevented by replacement with 0.5ml of normal saline after each sampling. Serum was separated and stored at – 20º C till the analysis.

Evaluation of the effect noni juice/G. biloba on CYP2C9 Substrate

Male Wistar rats weighing 250 – 300 g were used for the study. Animals were maintained on regular rat feed of the animal house. The rats were divided into three groups of
6 animals each. The group 1 served as control. The group 2 was pretreated with 5ml/kg of noni juice for 7 days. The group 3 was pretreated with 100mg/kg of aqueous extract of *G. biloba* for 7 days. The rats were fasted for 16 hours prior to the study with water *ad libitum*. On day 8 the group 1 was administered orally with 50mg/kg of diclofenac sodium (suspended 0.1% Tween 20); group 2 was administered orally with 5ml/kg noni juice followed by 50mg/kg of diclofenac sodium after a gap of 30min; group 3 was administered orally with 100mg/kg of *G. biloba* followed by 50mg/kg of diclofenac sodium after a gap of 30min. Blood samples were withdrawn by the retro - orbital vein puncture at different time intervals, viz. 0, 2, 4, 6, 8, 12 and 24 hours. The hypovolaemic condition was prevented by replacement with 0.5ml of normal saline after each sampling. Serum was separated and stored at – 20°C till the analysis.

**HPLC analysis of Phenytoin**

The serum concentrations of the phenytoin were determined by the HPLC assay method reported by Kishore *et al.*, 2003. [45] Stock solution of phenytoin (1mg/ml) was prepared in methanol, which was further diluted with methanol to the required concentrations viz., 0.5, 2.5, 10.0 and 50.0µg/ml of phenytoin. A standard graph was prepared by adding known concentration of phenytoin to drug free rat serum. Briefly, to each 100µl of serum sample, 100µl of each standard drug concentration and 20µl of internal standard stock solution was further diluted with methanol to the required concentrations viz., 0.1 – 50.0µg/ml. A standard graph was prepared by adding known concentration of diclofenac sodium to drug free rat serum. Briefly, to each 100µl of serum sample, 50µl of each standard drug concentration and 50µl of chlorzoxazone (internal standard 5µg/ml dissolved in methanol), 100µl of 2M HCl were added and vortex mixed for 3min. Then 3ml of chloroform was added and vortex mixed for 5min followed by centrifugation for 10 min at 3000rpm. The organic layer was separated, evaporated to dryness and reconstituted with 100µl of mobile phase and 20µl of the sample was injected onto HPLC. The mobile phase consisted of a mixture of Methanol: Ammonium Acetate Buffer (0.1M, pH4.2 adjusted with glacial acetic acid): Acetonitrile (60:30:10 v/v/v). The mobile phase was degassed using ultrasonic bath (Model Sonorex, Bandelin Electronic, Germany). The analysis was performed isocratically at a flow rate of 1ml/min and detector was operated at a wavelength of 280 nm. The data analysis was performed by LC Solution software (Shimadzu Corporation, Kyoto, Japan).

**Pharmacokinetic analysis**

Non compartmental pharmacokinetic analysis was carried out using the Kinética TM software (Version 4.4.1, Thermo Electron Corporation, USA). The following pharmacokinetic parameters were calculated: $C_{max}$, observed maximum serum concentration for each serum sample; $T_{max}$, sampling time of the maximum serum concentration; $t_{1/2}$, terminal elimination half life; AUC$_{0-t}$, area under serum concentration/time plot until the last quantifiable value; AUC$_{0-inf}$, area under serum concentration/time plot extrapolated to infinity, MRT, mean residence time of drug.

**Determination of oxidative stress markers**

The rats were divided into 5 groups of six animals each. All animals were treated for a period of 30 days. Animals in group I served as control (untreated), animals of group 2 were treated with 20mg/kg of phenytoin, animals in group 3 were treated with 5ml/kg of noni juice and animals in group 4 were treated with 5ml/kg of noni juice and 20mg/kg ofphenytoin. Animals of group 5 were animals were treated with 100mg/kg of *G. biloba*, animals in group 6 were treated with 100mg/kg of *G. biloba* and 20mg/kg of phenytoin. Blood samples were collected from the rats by retro orbital vein puncture at 5 day intervals; serum samples were separated and used for the determination of total antioxidant status and lipid peroxide levels.

**Determination of total antioxidant status**

The total antioxidant status in serum samples was determined by using DPPH method of Reddy *et al.*, 2004. [47] Ascorbic acid was used as a reference standard. The standard graph was prepared using different concentrations of ascorbic acid in water and the antioxidant status values were expressed in terms of nM of ascorbic acid.
Determination of lipid peroxides

The lipid peroxides in serum were measured by the method of Ohkawa et al., 1979. The standard graph for determination of malondialdehyde levels was prepared using 1,1,3,3 - tetraethoxy propane (TEP) reagent as the standard and the MDA content in the serum was expressed in nm/ml.

Statistical analysis

All the means are presented with their standard deviation (mean ± SD). All the parameters were compared between the control and pretreated groups using one way ANOVA, followed by post hoc Dunnet test.

RESULTS AND DISCUSSION

The serum concentration – time profile of phenytoin in different groups of rats is shown in Fig 1 and the pharmacokinetic parameters are presented in Table 1. The data shows that there was a significant decrease in the peak concentration ($C_{max}$) and bioavailability of phenytoin in noni juice pretreated group where as they were increased in case of G. biloba pretreated rats when compared with the control group. There was 3.0 and 3.20 fold decrease in $C_{max}$ of phenytoin in group 2 and group 4, whereas there was 1.49 fold increase in group 3 and 1.44 fold decrease in group 5 respectively. The AUC$_{total}$ and AUC$_{0 to n}$ of phenytoin in noni juice pretreated group decreased to the extent of 2.81 and 4.02 fold respectively when compared to control. Where as the AUC$_{total}$ and AUC$_{0 to n}$ in G. biloba pretreated rats increased to the extent of 2.08 and 1.59 fold when compared to control. The AUC$_{total}$ and AUC$_{0 to n}$ of phenytoin in rifampicin pretreated group decreased by 5.25 and 7.04 fold when compared to control. The AUC$_{total}$ and AUC$_{0 to n}$ of phenytoin in fluconazole pretreated group increased by 1.09 and decreased by 1.26 fold when compared to control. The terminal half – life ($t_{1/2}$) in group 2, group 3, group 4 and group 5 was found to be decreased by 2.86, 2.63, 5.92 and 1.93 fold. The Mean Residence Time (MRT) in group 2, group 3, group 4 and group 5 was found to be decreased by 1.47, 1.19, 2.44, 1.10 fold. The $t_{max}$ in the control and noni juice pretreated groups remained unaltered, while it was increased to 4h in G. biloba pretreated rats.

The serum concentration – time profile of diclofenac sodium is shown in Fig 2 and the obtained pharmacokinetic parameters are presented in Table 2. The present study also demonstrates that there is a significant decrease in the peak concentration ($C_{max}$) and bioavailability of diclofenac sodium (a typical CYP2C9 substrate) in noni juice pretreated group, where as there was a significant increase in case of the G. biloba pretreated rats when compared with the control group. There was 1.81 fold decrease in $C_{max}$ of diclofenac sodium in noni juice pretreated group, where as there was 1.77 fold increase in $C_{max}$ of G. biloba pretreated rats. The AUC$_{total}$ and AUC$_{0 to n}$ of diclofenac sodium in noni juice pretreated group decreased to the extent of 2.92 and 2.71 fold when compared to control.
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**Table 1: Pharmacokinetic parameters following oral administration of phenytoin in different groups of rats**

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Control group</th>
<th>Noni juice pretreated group</th>
<th>Ginkgo biloba pretreated group</th>
<th>Rifampicin pretreated group</th>
<th>Fluconazole pretreated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (μg/ml)</td>
<td>11.69 ± 0.61</td>
<td>3.86 ± 0.98*</td>
<td>17.45 ± 1.51*</td>
<td>3.65 ± 0.49*</td>
<td>8.11 ± 0.49*</td>
</tr>
<tr>
<td>$AUC_{\text{total}}$ (μg/ml/h)</td>
<td>45.76 ± 2.03</td>
<td>16.27 ± 1.76</td>
<td>96.07 ± 3.68*</td>
<td>8.28 ± 0.51*</td>
<td>50.24 ± 2.10</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (μg/ml/h)</td>
<td>55.72 ± 4.19</td>
<td>13.84 ± 1.44*</td>
<td>88.70 ± 3.97*</td>
<td>7.91 ± 0.54*</td>
<td>44.21 ± 2.26</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>7.11 ± 1.52</td>
<td>2.48 ± 0.34*</td>
<td>2.70 ± 0.42*</td>
<td>1.20 ± 0.08*</td>
<td>3.67 ± 0.03</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>7.03 ± 1.20</td>
<td>4.78 ± 0.38*</td>
<td>5.88 ± 0.41</td>
<td>2.38 ± 0.10*</td>
<td>6.38 ± 0.17</td>
</tr>
</tbody>
</table>

Values are Mean ± SD (n=6); *p < 0.05 compared to control

The total antioxidant status and lipid peroxide levels of the different groups of animals is shown in Fig. 3 and Fig. 4.

The present investigation brings to light that there is significant herb – drug interaction between noni juice/*G. biloba* with the commonly prescribed antiepileptic drug phenytoin, which is metabolized by CYP2C9 enzymes primarily to 5-(p-hydroxyphenyl)-, 5-phenylhydantoin (HPPH). The results show that noni juice decreased the bioavailability of phenytoin while *G. biloba* increased the...
bioavailability of phenytoin. Since there is significant difference in $t_{1/2}$ of noni juice/G. biloba pretreated rats when compared to control the possibility of involvement of intestinal absorption, intestinal metabolism and hepatic metabolism exists. The involvement of P-glycoprotein (P – gp) in noni juice pretreated rats may be ruled out because phenytoin is a weak inhibitor of p-gp.\textsuperscript{[50]} If the involvement of P-gp is speculated then the bioavailability of phenytoin should have been increased but in the present study the bioavailability of phenytoin is decreased. Hence

Figure 3: Total antioxidant status in rats of four different groups: All groups are treated for 30 days. CONT: control group (untreated); PHE: treated with phenytoin; NJ: treated with noni juice; NJPHE: treated with noni juice and phenytoin; GB: treated with G. biloba; GBPHE: treated with G. biloba and phenytoin. Total antioxidant status was measured in nM/ml of Ascorbic acid. Data are presented as mean ± standard deviation (SD) n = 6 for each group.

Figure 4: Serum lipid peroxides (MDA) levels in different groups of rats: All groups are treated for 30 days. CONT: control group (untreated); PHE: treated with phenytoin; NJ: treated with noni juice; NJPHE: treated with noni juice and phenytoin; GB: treated with G. biloba; GBPHE: treated with G. biloba and phenytoin. Lipid peroxidation was measured in nM/ml of MDA. Data are presented as mean ± standard deviation (SD) n = 6 for each group.
the involvement of intestinal and hepatic CYP2C9 induction might be the possible mechanism for the decrease in the bioavailability of phenytoin. Further, the results also show that the % reduction in bioavailability of phenytoin in rats pretreated with noni juice is almost similar to the % reduction in bioavailability of rats pretreated with CYP2C9 inducer (rifampicin), which indicates that noni juice has similar type of inductive effect on CYP2C9 enzyme. Till date, there are no reports on the interaction potential of noni juice with drugs except that it increases the coumadin resistance due to the presence high amount of potassium ions in it. The actual constituents responsible for the observed interaction cannot be specified because the noni juice contains several phytoconstituents. The exact mechanism by which noni juice modulates phenytoin metabolism is currently unknown because the fruit juices, alcoholic beverages, teas, and herbal extracts are complex chemical mixtures and it is very difficult to determine which compound or compounds are responsible for the potential interaction with metabolizing enzymes leading to herb – drug interactions.[51] The phenolic functional micronutrients of noni juice such as damnacanthal, scopoletin, morindone, alizarin, aucubin, nordamnacanthal, rubiadin, rubiadin-1-methyl ether and other anthraquinone glycosides might have led to the present interaction. As most of the anthraquinone derivatives have laxative effect, it may be speculated that anthraquinones present in noni juice might have lead to increased elimination rate of phenytoin. There is also a possibility that the anthraquinone derivatives and other active constituents of noni juice may lead to induction of hepatic microsomal enzymes. In case of G. biloba pretreated rats the % increase in the bioavailability of phenytoin is similar to the % increase in bioavailability of rats pretreated with flucanazole (CYP2C9 inhibitor) and the increase in bioavailability of phenytoin can also be attributed to inhibition of P-glycoprotein (P-gp) because phenytoin is known to be a weak inhibitor of P-gp. G. biloba is reported to alter the pharmacokinetics of theophylline by induction of CYP1A2, propranolol by induction of CYP2B1/2 and CYP3A1.[52-53] G. biloba extract is proven to reduce the therapeutic potency of phenobarbital due to induction of CYP2B enzymes.[54] Shinozuka et al., 2002[55] reported that feeding of G. biloba extract for 4 weeks significantly reduced the hypotensive effect of nicardipine which is metabolized by CYP3A4 in rats. Yoshioka et al., 2004[56] reported that G. biloba extract significantly inhibits the metabolism of nifedipine which is a typical CYP3A4 substrate. Sugiyama et al., 2006[57] reported that pretreatment with G. biloba extract significantly reduced the hypoglycemic action of tolbutamide in elderly rats due to induction CYP2C9. Umegaki et al., 2002[58] reported that pretreatment of rats with G. biloba increased the concentration and activity of various CYP enzymes (containing CYP1A1, CYP1A2, CYP2B, CYP2E1 CYP3A and CYP2C9) in the rat liver. The studies of Gaudineau et al., 2004[59]; He and Edeki 2004[60] and Numa et al., 2007[61] proved that the active constituents of G. biloba have CYP2C9 inhibitory potential and the present results are in agreement with these reports. In contrast, our results are in contradictory to the results of Sugiyama et al., 2004 and Umegaki et al., 2002. The actual constituents responsible for the increased bioavailability of phenytoin in G. biloba pretreated rats cannot be specified because it contains 30 different flavonoid compounds.[62] In both the noni juice and G. biloba pretreated rats the same type inhibitory and inductive effect was observed on the pharmacokinetics of diclofenac sodium (a typical CYP2C9 substrate). Hence it may be speculated that noni jucie has inductive effect where as G. biloba has inhibitory effect on CYP2C9. The total antioxidant status in the rats pretreated with phenytoin alone decreased significantly when compared rats co - administered with noni juice and phenytoin/aqueous extract of G. biloba and phenytoin. The lipid peroxide levels were found to be more in the rats pretreated with phenytoin alone when compared rats co - administered with noni juice and phenytoin/aqueous extract of G. biloba and phenytoin. These results clearly show that the polyphenolic constituents of noni juice and the flavonoids present in the G. biloba aqueous extract offer significant protection against the oxidative stress induced by long term administration of phenytoin.

In conclusion it is observed that there is significant herb – drug interaction between noni juice/ G. biloba with phenytoin. However, additional studies are required to define which active constituents in noni juice/G. biloba are responsible for induction and inhibition of CYP isozymes.

ACKNOWLEDGEMENTS

The authors wish to thank University Grants Commission, New Delhi for providing the financial assistance to carry out this work.

REFERENCES


